COMPARATIVE OSTEOHISTOLOGICAL ANALYSIS OF TWO MESOPREDATORS *DIDELPHIS VIRGINIANA* AND *PROCYON LOTOR*

By

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COMPARATIVE OSTEOHISTOLOGICAL

ANALYSIS OF TWO MESOPREDATORS

DIDELPHIS VIRGINIANA AND *PROCYON LOTOR*

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Title of Study: Comparative osteohistological analysis of two mesopredators, *Didelphis virginiana* and *Procyon lotor*

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Abstract: Examining the bone microstructure of modern vertebrates can reveal information about the biology and environment of extinct animals. *Didelphis virginiana*, the Virginia opossum, and *Procyon lotor*, the common raccoon, are both mesopredators that inhabit a wide variety of environments in North America. These two animals are omnivorous, nocturnal, and arboreal, exploiting similar resources in both urban and rural settings. However, the opossum, a marsupial mammal, has a much lower metabolic rate than the raccoon. This study qualitatively analyzes the osteohistology of these two mammals and adds to a growing database of modern vertebrate osteohistological descriptions. The right and left humerus, ulna, radius, femur, tibia, and fibula of an opossum and female raccoon collected from Tulsa County, Oklahoma, were histologically sectioned. The left side of the dentary of both animals was sectioned as well. The epiphyses of both animals were found to be unfused indicating they are both juveniles. The bone microstructure of contralateral elements for each individual was extremely similar. The opossum elements exhibit a high proportion of poorly organized parallel-fibered bone with mostly radial vascularization, which is remarkably scarce. The cortex of the raccoon bones are mainly fibrolamellar tissue with longitudinal vascularization that is much denser than seen in the opossum. No lines of arrested growth (LAGs) were found in the raccoon elements, indicating the animal was less than a year old. One LAG was found in the opossum indicating the animal was at least a year old. These two mesopredators inhabited similar niches and grew to comparable sizes (based on limb length) yet their histology records different growth patterns. The poor vascularization of the opossum bones and abundance of parallel-fibered tissue indicates a slower growth rate, likely due to its lower metabolic rate. Previous paleontological research has hypothesized on the metabolic rates of extinct animals based on osteohistological patterns. This study shows that similarly sized animals may both be homeothermic, yet exhibit different histological patterns.

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CHAPTER I

HISTOLOGY DEFINITIONS

Bone formation is either endochondral or intramembranous. Most bones in the vertebrate skeleton are formed endochondrally, such as all long bones that are typically used to gather histological data on extinct and extant animals. Endochondral bone formation differs from intramembranous bone formation in requiring a cartilaginous precursor, which is subsequently replaced by bone tissue (Leboffe 2013). Ossification begins in the mid-diaphyseal region of the bone, spreading towards the epiphyseal ends of the bone (Junqueira 2005). A secondary ossification center later appears in the epiphysis, or end of the bone and fuses with the diaphysis when maximum length is reached.

According to Huttenlocker, Woodward and Hall (2013), on a microstructural level, bone consists of the mineralized extracellular matrix, which can provide information about the signals found in an individual bone. Lamellar bone consists of highly organized fibers deposited next to one another and appears as anisotropic swathes of tissue under polarized light. This type of tissue is deposited more slowly than other types of bone. Woven bone is comprised of disorganized collagen fibers and appears isotropic under polarized light (Ross and Pawlina 2006). Woven bone often contains primary or Haversian canals, which contain blood vessels and nerves in a living animal (Padian and Lamm 2013). Woven bone containing primary osteons is called fibrolamellar bone

(Huttenlocker, Woodward and Hall, 2013). Three cell types play important roles in the deposition, maintenance and remodeling of bone (Junqueira 2005). Osteoblasts secret bone matrix and osteoclasts resorb bone matrix. Resorption cavities appear as porosities with scalloped edges, as bone resorption is often uneven. Osteocytes are cells situated in the matrix in osteocyte lacunae (Ross and Pawlina 2006). Dense numbers of osteocyte lacunae indicate a rapid rate of growth, as a greater number of cells illustrate more activity in that area of the bone. Vascular canals permeate bone in order to provide nutrients, oxygen and transport for cells (Ross and Pawlina 2006). The orientation and density of vascular canals can provide information about how quickly bone was deposited during the growth of an organism. Faster growing bone is more vascularized, as more nutrients are required to sustain growth. In general, the more anastomosing observed in vascular canals, the faster a bone was growing (Huttenlocker, Woodward and Hall, 2013). Lines of arrested growth (LAGs) are deposited yearly in many animals and can be used to estimate an annual rate of growth. LAGs can be easily obscured, however, by factors such as medullary drift, remodeling, or broken bone (Huttenlocker, Woodward and Hall, 2013). Medullary drift occurs when bone is resorbed from the endosteal surface and subsequently re-deposited in another area of the endosteal circumference, revising the shape of the bone. The external fundamental system (EFS) is an accumulation of LAGs at the periosteal margin of the bone as growth slows and LAGs are laid down closely together. According to Amprino's rule, the rate at which bone is deposited determines most of the observed histological characteristics (Amprino 1947). Another factor that contributes to histological arrangement is mechanical stress. Wolff's Law states that mechanical stresses on bone are one of the most decisive influences (Wolff 1892).

INTRODUCTION

The bone microstructure of extant vertebrates provides understanding of fossil animal physiology, taxonomy, pathology, and general life history, among other data (Padian 2013). For

example, according to Horner et. al (2000), the osteohistology of the dinosaur *Maiasaura peeblesorum* reveals growth rates and separate ontogenetic stages for *Maiasaura peeblesorum*. The current study examines the osteohistology of the extant North American opossum, *Didelphis virginiana*, and the North American raccoon, *Procyon lotor*, in order to make a comparison between the histological narratives recorded in the long bones and dentary of two similarly sized mammals, one marsupial and one placental, respectively. These two animals occupy similar ecological niches as omnivorous nocturnal opportunists, yet they have different metabolic rates (Austad 1997, Brocke 1970, Johnson-Delaney 2014, Mugaas 1993). This study investigates whether two animals occupying similar niches, but employing divergent metabolic strategies, exhibit a comparable history of bone growth at a microstructural level, although some differences in bone microstructure will most likely be attributable to phylogenetic signal (Padian 2013), as these two animals are not closely related.

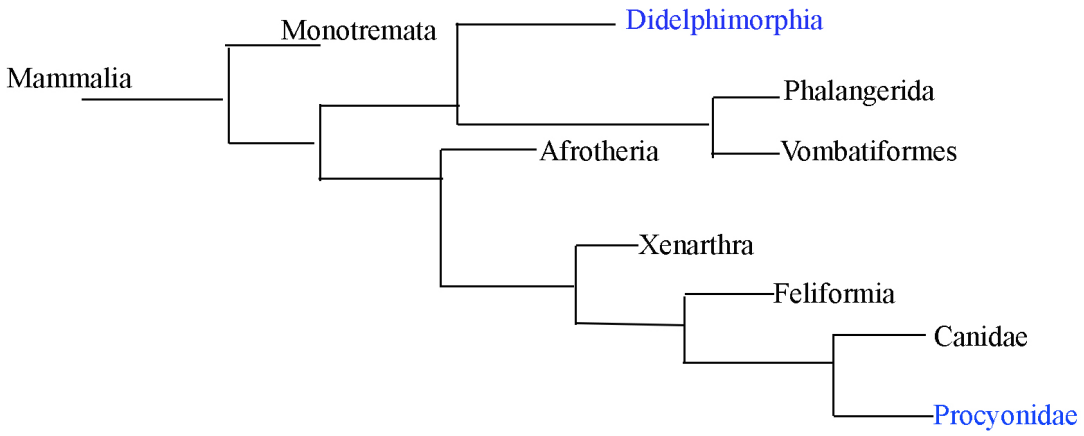

Fig. 1 Cladogram of the familial relationship of *Didelphis virginiana* and *Procyon lotor (after Gallus 2015, Koepfil 2007, Tarver 2016)*

Figure 1 is a cladogram showing the relationship of *Didelphis virginiana* (Didelphimorphia), a member of the order Didelphimorphia, and *Procyon lotor* (Procyonidae), a member of the family Procyonidae at the family level. These two mammals are not closely related, as shown in the cladogram.

The North American raccoon, *Procyon lotor*, and the North American opossum, *Didelphis virginiana*, are both medium-sized, omnivorous mesopredators. Mesopredators are mammalian predators that occupy the midlevel in a food web and tend to weight between 1 and 15 kg (Buskirk 1999). These two taxa share other characteristics as they exploit similar resources in their environments, which sometimes overlap (Ladine 1997). Both species have been successful in urban areas, partly due to their omnivorous diets, medium body sizes, and behavioral flexibility (Bateman 2012). As these two animals have similar life histories, they provide an opportunity to examine the similarities and differences in bone histology in two animals that exploit similar niches but are phylogenetically separated and have different metabolic rates. Whereas paleontologists have historically been left to speculate about the relationships between metabolism, phylogeny, and growth (Grady 2014, Thomas 1980, Paladino 1990, Spotila 1991, Ruben 1996), modern analogues now allow us to examine the bone microstructure of animals with known, different metabolic rates and observe how this difference in physiology affects bone growth. This study also aims to contribute to the growing database of modern vertebrate bone microstructure. Little work has been done on the bone histology of procyonids, or marsupials in general. Kolb et. al 2015 examined the white-eared opossum *Didelphis albiventris* and the latrine opossum *Lutreolina crassicaudata*. They found that the cortex of long bones was predominantly comprised of parallel-fibered bone, but woven-fibered was identified closer to the inner cortex. They found that the cortex is well vascularized with mainly longitudinal canals. They did not observe any LAGs in the specimens they examined. Enlow and Brown (1958) made similar observations about *Didelphis*. Werning (2013) asserts that

small placentals and small marsupials have a common histology not seen in larger marsupials and placentals, consisting of poorly vascularized lamellar bone, indicating this is a shared characteristic of therian mammals and relates to body size.. In contrast to *Didelphis*, the histology of *Procyon lotor* was examined once, by Enlow and Brown (1958), who found the cortex to be primarily reticular and radial primary bone.

The radiation of Procyonidae occurred during the Miocene in North America (Darlington, 1957) producing many arboreal and omnivorous species (Eisenberg 1981, Martin 1989). Over half of the 18 species that compose the family Procyonidae are found in the tropical regions of North and South America (Mugaas 1993), suggesting that procyonids in general are more suited to warmer climates. In contrast to this general trend, the North American raccoon has a higher basal metabolism than related procyonids, permitting it to inhabit regions beyond the tropical and subtropical ranges that bound others of its family (Mugaas, 1993). This provides the common raccoon with greater physiological flexibility than other procyonids (Mugaas 1993), allowing it to expand its northern range over time as well as thrive in a wide variety of habitats (Whitney and Underwood 1952).

Raccoons are nocturnal animals primarily found in deciduous and mixed forests, although they have quickly adapted to urbanization and are considered a particularly bothersome pest in many cities (Prange et al 2003, Riley et al 1998). Due to factors such as the eradication of large predators, an increase in agriculture and the spread of urbanization, raccoon populations in North America have exploded since the 1940s (Zeveloff 2002). In the wild, raccoons thrive in areas that contain trees that are easy to climb, as well as abundant water sources (Heske 2016). Raccoon densities are also affected by the abundance of suitable den locations (Beasley 2007). While these may vary from tree cavities to log piles and rock dens, the cavities of large trees are highly preferred (Beasley 2007). Male raccoons are more solitary than females, maintaining relatively large home ranges that do not overlap, while females have smaller home ranges that greatly

overlap (Kamler 2003). Raccoons live for approximately 2–3 years in the wild (Austad 1997), while captive raccoons can live for more than 20 years (Hohmann and Bartussek, 2001).

Didelphis virginia, or the Virginia opossum, is the only marsupial of 334 extant species that can be found in North America (Cardillo 2004). The remains of the Virginia opossum have been found in South America as early as the Pliocene, but have not been found in North America before the Pleistocene (Hibbard et. al 1965). Opossums have a lower resting metabolism than raccoons, which restricts their habitat to the southern part of North America (Kanda, 2005), although in the last century their range has expanded considerably northward and westward (McManus 1974). Similar to raccoons, opossums are nocturnal omnivores that prefer a forested landscape, although they are quite successful in a variety of environments including arid regions and urban landscapes(McKinney 2002). Nonetheless, opossums prefer landscapes supplied with plentiful water sources much like raccoons (McManus 1974). Their ranges have been observed to be elongate rather than round as they follow water sources such as streams (Llewellyn and Dale, 1964).

Opossums have a much lower metabolic rate than many mammals, recorded at 0.15 ml O_2/g of body weight/hr for a 3.5kg individual (Brocke 1970). The lower critical temperature is around 25°C (Brocke 1970). The average body temperature for opossums is around 35.2°C (Higgenbotham and Koon, 1955). In comparison, the raccoon has a much higher metabolism than the opossum, at 0.54 ml O_2/g of body weight/hr (Mugaas 1993).

Sexual dimorphism is seen in opossums with males being slightly larger and weighing more (Hamilton 1958). Although the life span of the opossum was once thought to be up to 7 years (Hartman 1923), recent observation puts the maximum lifespan closer to 4.5 years (Austad 1997) and the average lifespan in the wild at approximately 2 years (Johnson-Delaney 2014). Sexual maturity is reached at about the age of 8 months (Austad 1997).

This study attempts to qualitatively describe the bone microstructure of *Didelphis virginiana* and *Procyon lotor*, two medium-sized mammals that occupy similar niches, but have different metabolic rates. In the field of paleontology, many hypotheses regarding the metabolism and growth patterns of extinct animals would benefit from the analysis of modern vertebrate analogues. This study also aims to contribute to the growing database of modern vertebrate osteohistological descriptions.

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CHAPTER II

MATERIALS AND METHODS

A female raccoon, *Procyon lotor*, and an opossum, *Didelphis virginiana*, were used in this study. The sex of the opossum could not be determined as it was found in a desiccated state. Epipubic bones could not be viewed as an indicator of sex as these are found in both male and female marsupials. Both were deceased prior to collection in Tulsa County under Oklahoma Department of Wildlife Conservation permit number 6773. The raccoon was skeletonized using a dermestid beetle colony, while the opossum was discovered as a desiccated corpse upon collection and skeletonized by hot water maceration with Tergazyme detergent. Upon skeletonization of both specimens, it was discovered that neither had fused epiphyses in the long bones. This classifies both animals as juveniles (Sumner‑Smith 1966).

The length and least circumference of all elements were measured. All skeletal elements were then drawn and photographed using an iPhone 5c (Apple Inc., Cupertino, CA, USA), an iPhone 7, or a Cannon EOS Rebel T2i camera (Cannon Inc., Tokyo, JPN) prior to beginning the embedding process. All skeletal elements were drawn from the lateral and medial views. The right and left humerus, radius, ulna, femur, tibia, and fibula of the raccoon were used to construct an intraskeletal narrative for this animal. The left side of the dentary was included as well. The protocol established in Schweitzer et. al. 2006 for thin section slide production was followed for this study with some deviations. The bones were fixed in 10% formalin for two days before being washed in a 10% solution of Tergazyme for five days to remove tissue and fluids. All *Procyon*

samples then went through an ethanol dehydration series starting at 70% ethanol for seven days. Small transverse sections were cut from the long bones including the point of least circumference using the Buehler IsoMet 1000 6-inch diameter precision saw (Buehler, Lake Bluff, IL, USA) in order to avoid embedding the entire bone. Due to the morphology of the raccoon and opossum long bones, the area of least circumference did not always occur close to the mid-diaphysis. In some cases the bones were sectioned quite close to the proximal or distal end of the bone. A section was not removed from the dentary, as the shape did not lend itself to this method of embedding. The whole dentary and long bone sections were then put into 80% ethanol for three days and then 100% ethanol for seven days. These specimens were then cleared for 30 minutes using Clear Advantage, a xylene substitute, to remove any remaining fats and oils that might obscure the final thin section.

The dentary and long bone sections were embedded in Buehler EpoThin 2 epoxy resin and left in a refrigerator for 24 hours to slow the reaction and prevent heat damage to the specimen. They were then moved to a fume hood for another 24 hours until completely firm. The resin used to embed the raccoon jaw did not set, so it was heated in an oven at 60°C for 10 hours to speed up the catalyzation of the resin reaction. This did not succeed in setting the resin so the jaw was removed and re-embedded. The embedded blocks containing the long bone sections and dentary were cut on the line of least circumference using the Buehler IsoMet 1000 precision saw. The block was allowed to dry and penetrant stabilizer, followed by DevCon 5 minute epoxy glue, was applied to the exposed bone at the surface. Twenty-four hours later, a second cut was made in order to make several thin sections approximately two millimeters thick.

Two sections were produced to allow for any errors or accidents that might occur during slide production. The block and sections were allowed to dry and DevCon 5 minute epoxy glue was applied. The plastic slides used to mount the wafers of embedded bone were smooth so frosting was performed in order to provide a more textured surface to adhere the wafers. Frosting

of the plastic slides used for mounting occurred on the Buehler EcoMet 4 lapidary wheel, with polishing paper at 600 grit (Metallurgical Supply Co. Inc., Shenandoah, TX, USA), 120rpm. Pre-mount grinding of the two millimeter thick sections began at 600 grit until the surface was even and completely free of epoxy glue and then moved to 800 grit for a mirror finish. The wafers were then allowed to dry and mounted onto the frosted side of the plastic slides using Starbond medium cyanoacrylate glue (Starbond Inc., Los Angeles, CA, USA).

Slide mounting protocol followed Lamm (2013). After curing for 48 hours the slides were unwrapped and polishing commenced beginning at 180 grit until the thin sections were at a thickness of 750 microns, then moved up to 320 grit until the thickness was 500 microns, 600 grit until the thickness was 350 microns, and finally 800 grit until the thickness was 300 microns. The slides were then removed from the wheel and hand polished using South Bay Technology 5 micron precision alum abrasive powder and then South Bay Technology 1 micron alumina suspension gel (South Bay Technology Inc., San Clemente, CA, USA) to completely polish the slide and remove any obstructions to viewing the bone microstructure.

The opossum slides were made using a similar protocol with a few deviations. The bones were put into a 10% buffered formalin solution for two days and then moved to the ethanol series and the subsequent steps previously described for the raccoon leading to slide production. Opossum samples were not cleaned with Tergazyme after being fixed in formalin, as they were cleaned with Tergazyme while being skeletonized.

Pictures of all slides were taken with the Nikon Ri-2 camera attached to the Nikon Eclipse Ni-U polarizing microscope (Nikon, Corp., Tokyo, JPN) using an ASI motorized stage with glass plate insert. Slides were photographed under 10x and 5x objectives and under plane light, cross-polarized light and full wave plate light. Large photos were stitched together using several smaller photos in order to maintain a high level of magnification and detail in a large

photo. The software used to stitch together photos and process them is Nikon NIS-Elements: Documentation.

Histological terms used in qualitative descriptions follow Francillon-Vieillot et. al. (1990).

Element	Length (cm)
Raccoon	
Left Humerus	9.8
Right Humerus	9.8
Left Tibia	11.8
Right Tibia	11.7
Left femur	11.2
Right femur	11.3
Left radius	9.8
Right radius	9.7
Left ulna	11.5
Right ulna	11.5
Left fibula	12.3
Right fibula	12.3
Opossum	
Left humerus	6.2
Right humerus	5.9
Left tibia	7.1
Right tibia	7.0
Left femur	7.1
Right femur	7.1
Left radius	5.4
Right radius	5.4
Left ulna	7.1
Right ulna	6.8
Left fibula	7.1
Right fibula	7.0

Table 1. Length of raccoon and opossum long bones.

CHAPTER III

RESULTS

In both the opossum and the raccoon, the bone tissue microstructure of contralateral elements for each individual was nearly identical, so the histology of each element will only be described once. However, any discrepancies between the appearances of contralateral elements are also described.

Dentary

Raccoon

The raccoon dentary is shown under cross-polarized light in Figure 2. On the labial side towards the periosteal surface, bone is fibrolamellar and disorganized with moderate vascularization but few primary osteons. There are several large vascular canals in this area but osteocytes are not arranged around them. Closer to the alveolus there are many large resorption cavities, many of which merge with the mandibular canal. In the canal there are sparse trabeculae composed of lamellar bone. On the ventral side of the dentary there is a lamellar layer of bone on the endosteal surface. A clearly defined resorption line can be seen in Fig. 3, on the ventral side. Closer to the periosteal surface several lines can be seen, however these are unlikely to be LAGs or resorption lines as closer examination reveals them to be part of the lamellae of the

bone. Moving from the inner-cortex to the outer-cortex, there is a layer of compact coarse cancellous bone followed by a thick band of parallel fiber bone. The vasculature in this area is mainly radial with some longitudinal as well.

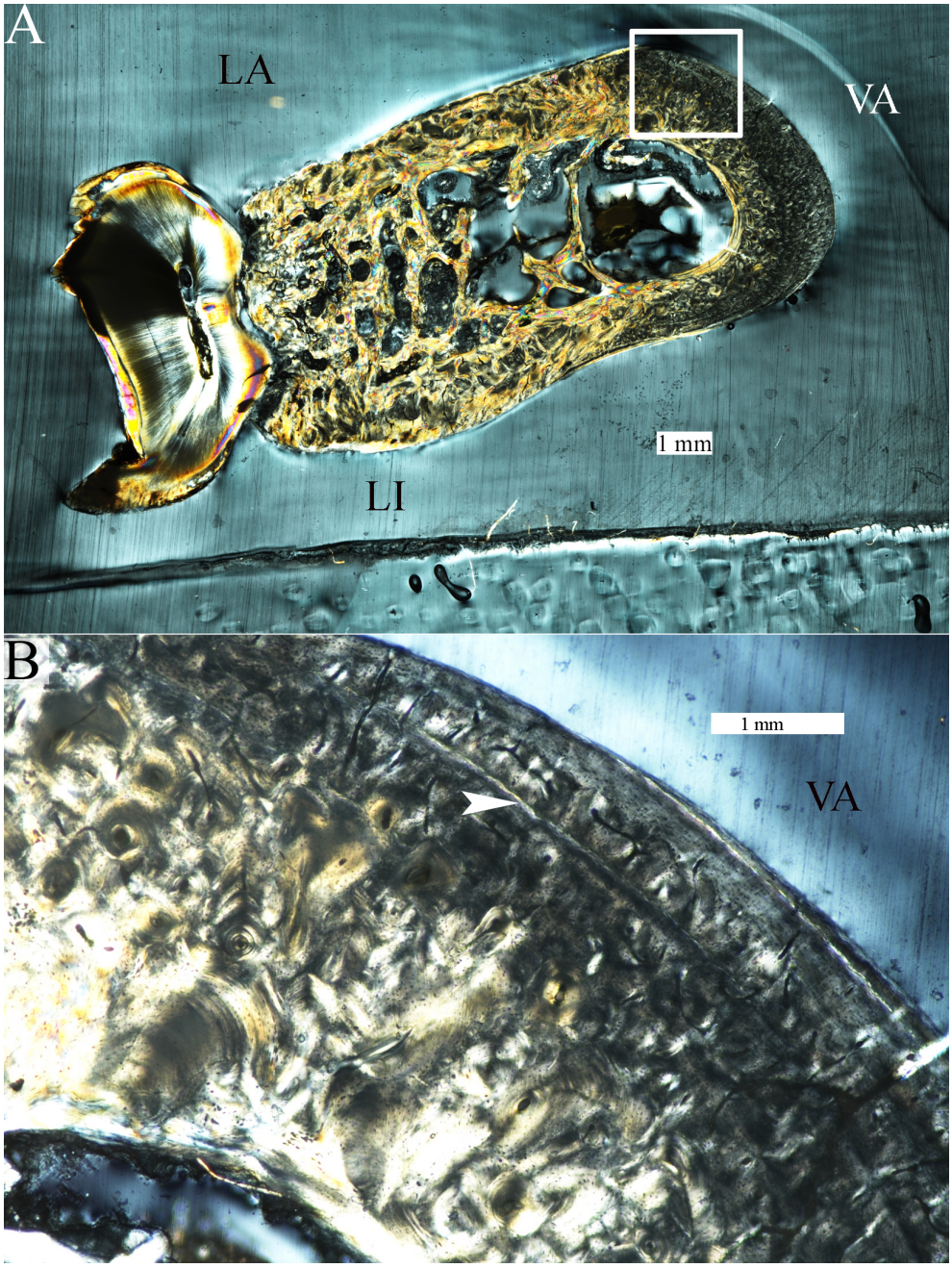


Fig.2 Microstructure of *Procyon lotor* dentary and resorption line.

(A) Raccoon dentary viewed under cross-polarized light shows the transition between lamellar bone layer along endosteal surface and primary bone comprising ventral side of the dentary. The area of the dentary in the white box is shown at greater magnification in B. LI- Lingual side of dentary. LA- Labial side of dentary. (B) This image shows the raccoon dentary viewed under cross-polarized light. The white arrow points to resorption line seen in dentary. VA- Ventral side of dentary.

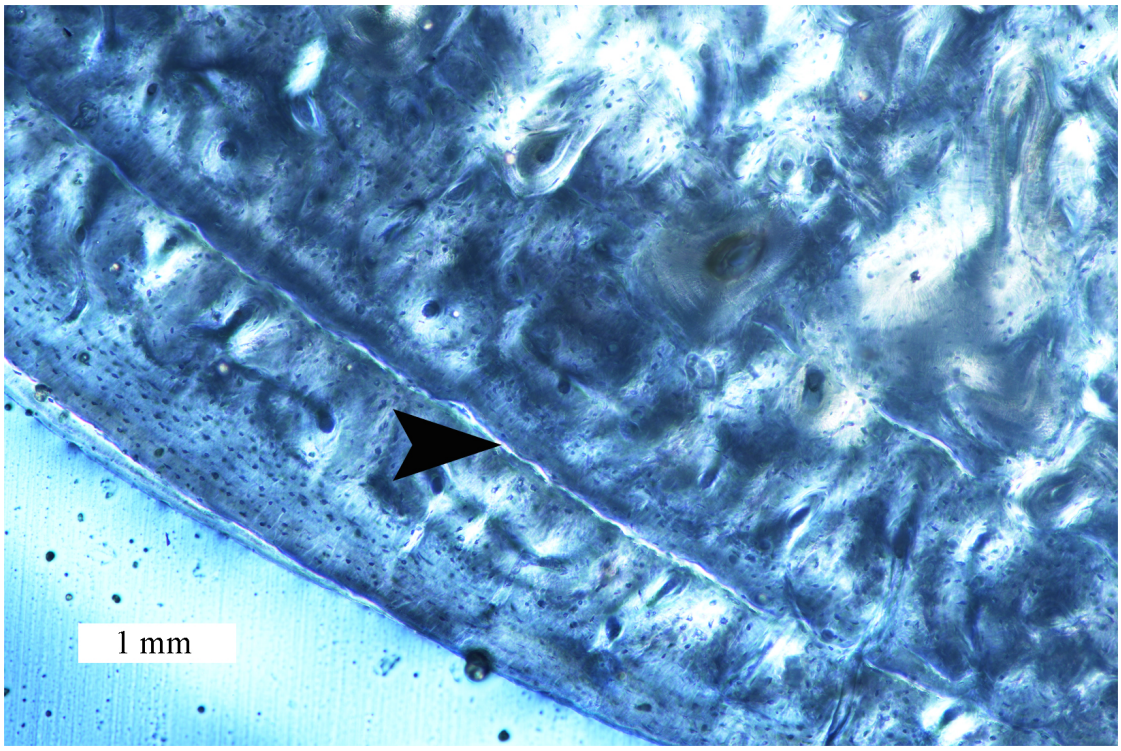


Fig. 3 Resorption line in *Procyon lotor* dentary.

This image shows the resorption line found in the raccoon dentary, which is shown by the black arrow. Due to the scalloped shape of the line, and the observation that it does not continue around the circumference of the bone, this is not a line of arrested growth.

Opossum

Figure 4 shows the opossum dentary under cross-polarized light. The labial side of the mandibular canal is partially filled with poorly organized parallel-fibered bone. There are alternating acellular swathes of bone and bone with dense osteocyte lacunae. From mid-cortex to outer cortex, the tissue is mostly avascular parallel-fibered bone. The ventral side has an endosteal layer of avascular lamellar bone with few osteocyte lacunae followed by a clear resorption line. The inner cortex then consists of fibrolamellar bone with longitudinal vascular canals. Some secondary osteons are present as well. The outer cortex is composed of avascular parallel-fibered bone with few osteocyte lacunae. The lingual side exhibits an endosteal layer of poorly organized parallel-fibered avascular bone with dense osteocyte lacunae. The endosteal surface exhibits some sparse trabeculae comprised of lamellar bone. The outermost cortex is avascular and acellular lamellar bone. Similar to the raccoon dentary, several lamellae can be seen at the periosteal edge most likely due to the angle at which the bone was sectioned rather than the presence of a LAG or resorption line. The dorsal side of the dentary has poorly organized parallel-fibered bone with dense osteocyte lacunae comprises the entire cortex. There is some

bacterial degradation in this area seen as large dark patches.

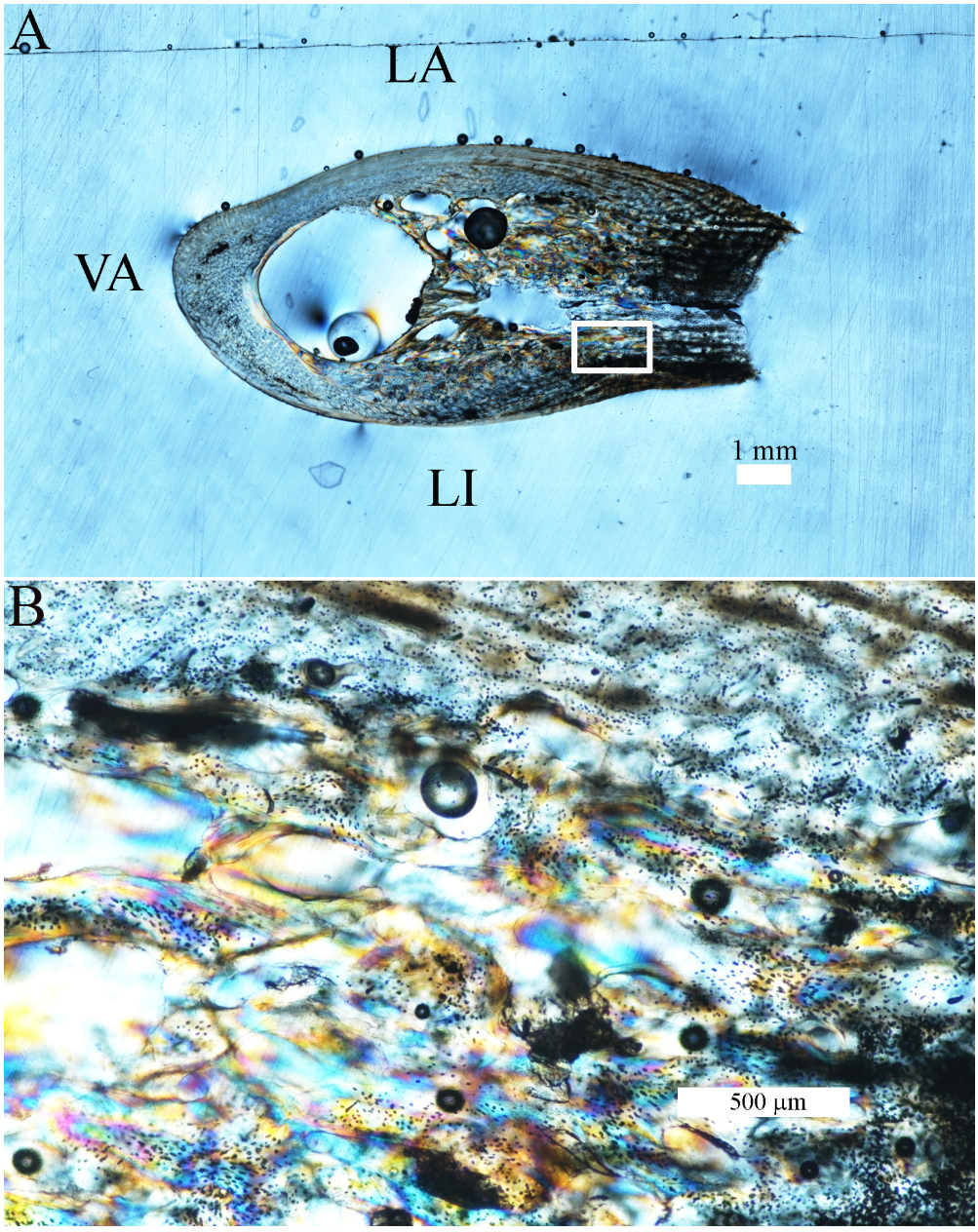


Fig.4 Microstructure of *Didelphis virginiana* dentary and osteocyte lacunae density.

(A) This image shows the left side of opossum dentary viewed under cross-polarized light. The area of bone in the white box is shown at greater magnification in B. LI- Lingual side of dentary. LA- Labial side of dentary. VA- ventral side of dentary. (B) This image of the opossum dentary shows alternating areas of acellular tissue and tissue with dense numbers of osteocyte lacunae.

Raccoon

Femur

Figure 5 (A) and (B) show the raccoon humerus under full wave plate light. The anterior side of the cortex of the left femur has a thin layer of lamellar bone on the endosteal surface. About half the cortex is compact coarse cancellous bone with simple vascular canals. There are a few primary osteons as well. This is followed by a layer of fibrolamellar bone with denser longitudinal vascularization. Close to the periosteal surface of the bone there is a layer of avascular parallel-fibered bone followed by a row of longitudinal vascular canals. A thin layer of avascular parallel-fibered bone lies at the periosteal surface. The lamellar bone on the posterior endosteal surface extends to mid-cortex in some places. Nearly the entire cortex on the posterior side is compact coarse cancellous bone with longitudinal and laminar vascularization. There is a layer of parallel-fibered avascular bone at the periosteal surface. The lateral side of the bone, from the inner cortex to past mid-cortex, has a higher proportion of lamellar bone which is mostly avascular with the exception of one large vascular canal forming a large primary osteon with bone fibers and osteocytes arranged circumferentially around the periosteal edge and linearly along the endosteal edge. A layer of compact coarse cancellous bone with some primary osteons extends from mid-cortex to the periosteal surface. The medial side of the endosteal surface has a layer of lamellar bone that appears fragmented, either due to some mechanical trauma during processing or unorganized resorption. There is some resorptive scalloping on the endosteal surface. Most of the medial cortex is compact coarse cancellous bone with longitudinal vascularization. There is a resorption line above this followed by a thin layer of fibrolamellar bone. A row of simple vascular canals and then a thin layer of parallel-fibered bone follow.

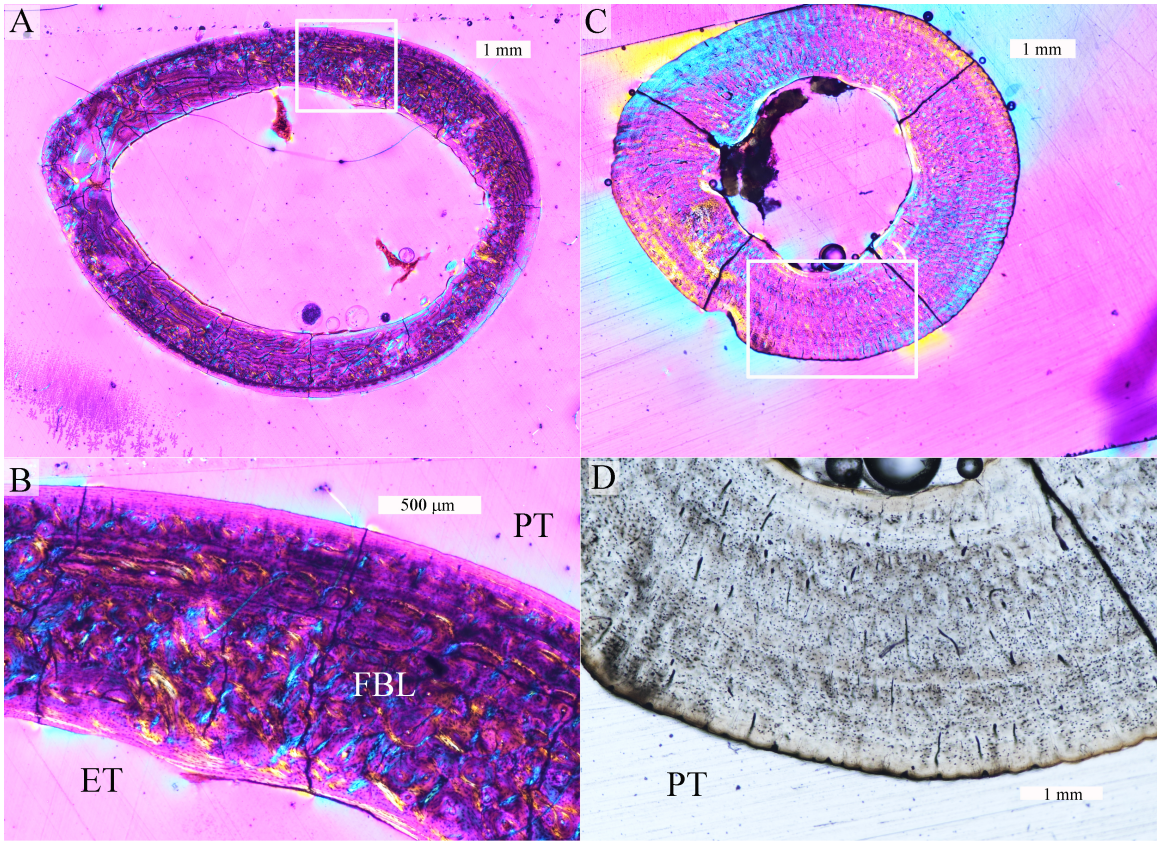


Fig.5 Microstructure of *Procyon lotor* and *Didelphis virginiana* femora.

(A) The left femur of the raccoon under full wave plate light shows a cortex composed primarily of fibrolamellar bone. This is shown in detail in B, which is taken from the area in the white box in A. (B) This is a closer view of the left femur of the raccoon under full wave plate light. The majority of the cortex is composed of fibrolamellar bone (FBL). ET indicates the endosteal surface of the bone, while PT indicates the periosteal surface of the bone. (C) This image shows the left femur of the opossum under full wave plate light. The cortex is nearly entirely parallel-fibered with some lamellar bone on the endosteal surface. The area of bone in the white box is magnified in D. (D) The left femur of the opossum is entirely parallel-fibered with radial vascularization shown here under plane light.

Tibia

Figure 6 (A) and (B) show the raccoon tibia under cross-polarized and plane light. The cortex of the left tibia from the inner cortex to the outer edge of the mid-cortex is compact coarse cancellous bone with longitudinal and reticular vascularization. A resorption line follows the compact coarse cancellous bone, as well as a layer of avascular parallel-fibered bone. Compact coarse cancellous bone comprises nearly the entire cortex on the anterior side. Some spicules of lamellar bone are seen in the medullary cavity on the endosteal surface. This pattern can also be seen on the lateral and medial sides of the bone. The anterior side includes a large vascular canal in the inner cortex surrounded by lamellar bone.

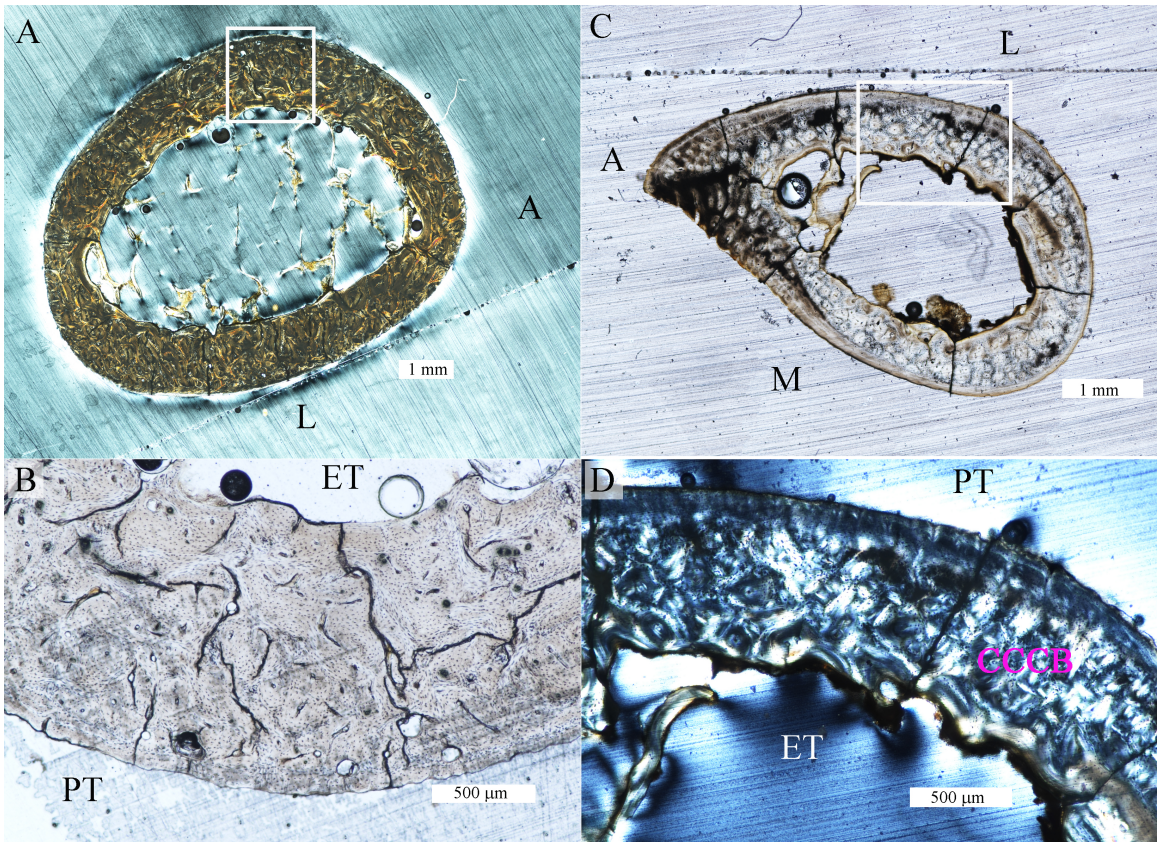


Fig.6 Microstructure of *Procyon lotor* and *Didelphis virginiana* tibiae.

(A) The left tibia of the raccoon, shown here under cross polarized light is mainly comprised of compact coarse cancellous bone. The white box in A is shown at greater magnification in B. A indicates the anterior side of the bone and L indicates the lateral side of the bone. (B) The

vascularization of the left tibia of the raccoon under plane light reveals longitudinal and reticular vascularization. ET indicates the endosteal surface of the bone while PT indicates the periosteal surface of the bone. (C) This image shows the opossum tibia under plane light. The area in the white box is magnified in D. (D) This image shows the opossum tibia under cross polarized light and highlights the compact coarse cancellous bone (CCCB) tissue that comprises the majority of the cortex.

Fibula

The raccoon fibula can be seen under full wave plate light in Figure 7 (A). There is a thin layer of lamellar bone around the endosteal surface of the left fibula. The remaining cortex is composed of compact coarse cancellous bone with longitudinal vascularization. Some well-defined osteons can be seen throughout the cortex, which is thickest on the lateral side. The left fibula exhibits a layer of lamellar tissue that extends to mid-cortex on the medial side. The rest of the cortex is comprised of compact coarse cancellous bone with simple vascularization and secondary osteons concentrated on anterior, lateral and posterior sides.

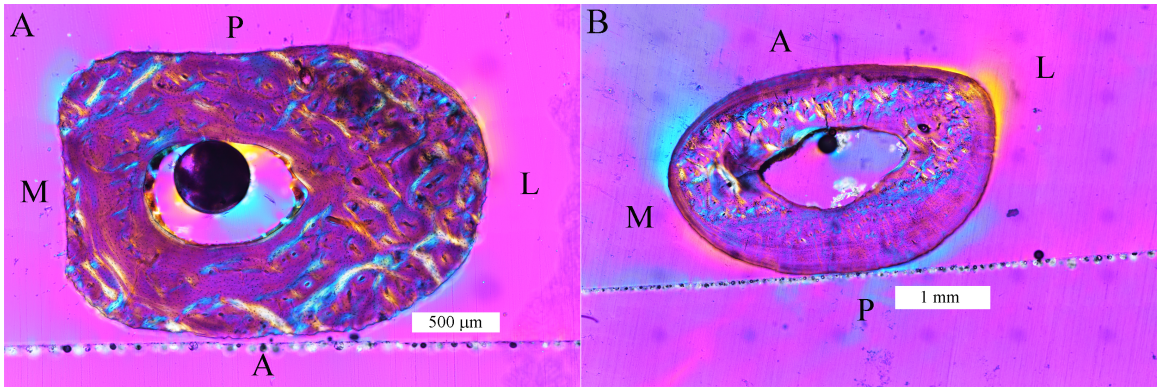


Fig.7 Microstructure of *Procyon lotor* and *Didelphis virginiana* fibulae.

(A) In this image of the left raccoon fibula, shown here under full wave plate light, compact coarse cancellous bone can be seen at the outer cortex as well as a thin layer of lamellar bone on

the endosteal surface. (B) Towards the anterior side of the left opossum fibula seen here under full wave plate light, the cortex is comprised mostly of compact coarse cancellous bone..Parallel-fibered bone is exhibited by the majority of the cortex on the posterior side of the bone.

Opossum

Femur

Figure 5 (C) and (D) show the opossum femur, with a focus on the sparse radial vascularization exhibited throughout the elements. The left femur has a thin layer of avascular lamellar bone on the endosteal surface. The cortex is primarily poorly organized parallel-fibered bone with sparse radial vascularization. There is a small depression on the periosteal surface of the anterior side of the bone. The anterolateral side has a small patch of loose parallel-fibered bone on the inner cortex that is oriented perpendicularly to the rest of the parallel-fibered bone. The right femur has similar microstructure. On the anteromedial side there is a layer of lamellar bone followed by a layer of loose parallel-fibered bone with longitudinal vascularization. From inner to mid-cortex there is a thin layer of parallel-fibered avascular bone. From mid-cortex to outer cortex the bone is loose parallel-fibered bone with radial vascularization. There are some signs of bacterial degradation in the inner cortex

Tibia

Figure 6 (C) and (D) show the opossum tibia under plane light and cross-polarized light. The left tibia has a thin layer of lamellar bone on the endosteal surface with some trabeculae protruding into the medullary cavity. The rest of the cortex is compact coarse cancellous bone, heavily obscured by bacterial degradation. On the posterior side, there is a layer of avascular lamellar bone. The outer cortex has a thin layer of avascular parallel-fibered bone. The vascularization is primarily longitudinal. There is a thin layer of avascular parallel-fibered bone at

the periosteal surface. The microstructure of the right tibia is similar to the left but there is more extensive bacterial degradation.

Fibula

Figure 7 (B) shows the opossum fibula under full wave plate light and Figure 8 shows a magnified view of the left fibula on the anterior side. The left fibula on the anteromedial side has some avascular lamellar bone followed by a layer of compact coarse cancellous bone with longitudinal vascular canals. From mid-cortex to outer cortex the bone is avascular parallel-fibered tissue. There is a layer of loose parallel-fibered bone on the posterior side, while the remaining cortex is comprised of avascular parallel-fibered bone. Most of the cortex of the lateral side of the bone is compact coarse cancellous bone with some longitudinal and a few radial canals. The outer cortex is avascular parallel-fibered bone. Some of the lamellae in the outer cortex on the posterior side are difficult to distinguish from LAGs/resorption lines, however, they do not have characteristics associated with either structure. The medial side has microstructure similar to the lateral side.

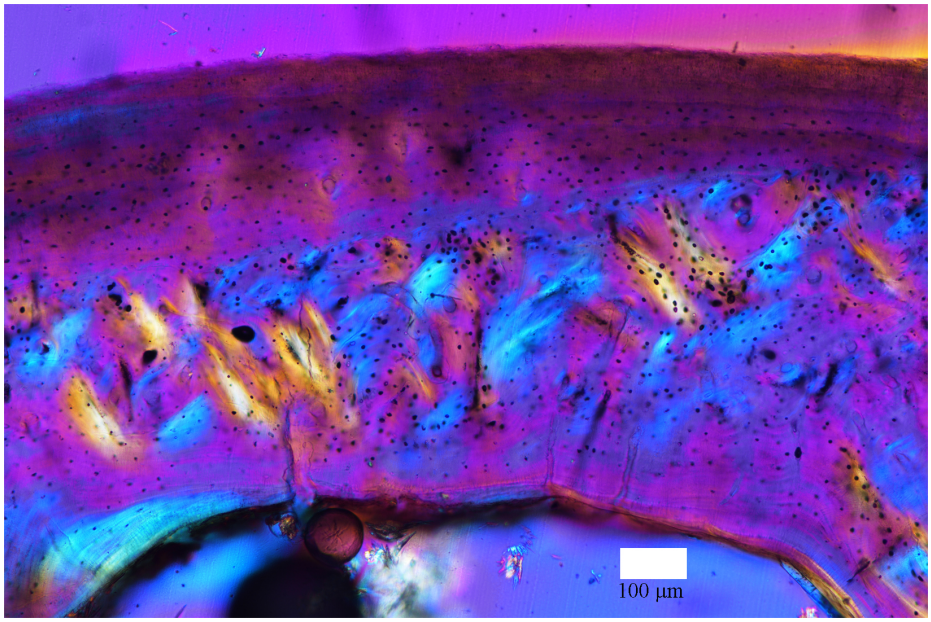


Fig. 8 Microstructure of *Didelphis virginiana* fibula.

This image shows a magnified view of Fig. 7 B, the left fibula of the opossum, taken on the anterior side of the cortex under full wave plate light. The striated swathes of bone are compact coarse cancellous bone that comprise the mid cortex on the anterior, medial and lateral sides of the bone.

Raccoon

Humerus

Figure 9 (A) and (B) show the raccoon humerus in cross-polarized and full plate light. There is a layer of lamellar bone on the anterior side of the left humerus, along the endosteal surface, which is mostly avascular, but a few primary osteons are present. Most of the cortex consists of fibrolamellar bone with some laminar canals and sparse primary osteons. A layer of parallel-fibered bone of varying thickness follows after this to the periosteal surface. It is vascularized by a few longitudinal canals. Posteriorly the lamellar endosteal tissue continues but thickens to comprise about one quarter of the cortex. It is populated by sparse primary osteons. There are some patches of compact coarse cancellous bone in this section as well. Fibrolamellar bone follows and forms most of the cortex. Vascularization is mostly laminar with some plexiform. Fibrolamellar bone is then followed by a layer of parallel-fibered bone, with longitudinal canals arranged in several linear bands. On the lateral side, the endosteal surface is scalloped due to resorption. There is fibrolamellar bone from innermost cortex to mid-cortex with mostly laminar canals, but some longitudinal as well. Parallel-fibered bone with some primary osteons extends from the mid-cortex to the periosteal surface. Medially there is a thin layer of lamellar bone on the endosteal surface followed by some patches of compact coarse cancellous bone. The mid to outer cortex of bone is fibrolamellar with reticular vascularization, which transitions to longitudinal vascularization closer to the periosteal surface. A thin layer of parallel-

fibered nonvascular bone lies at the periosteal surface. The periosteal surface is rough. Some periosteal attachment fibers can be seen close to the periosteal surface.

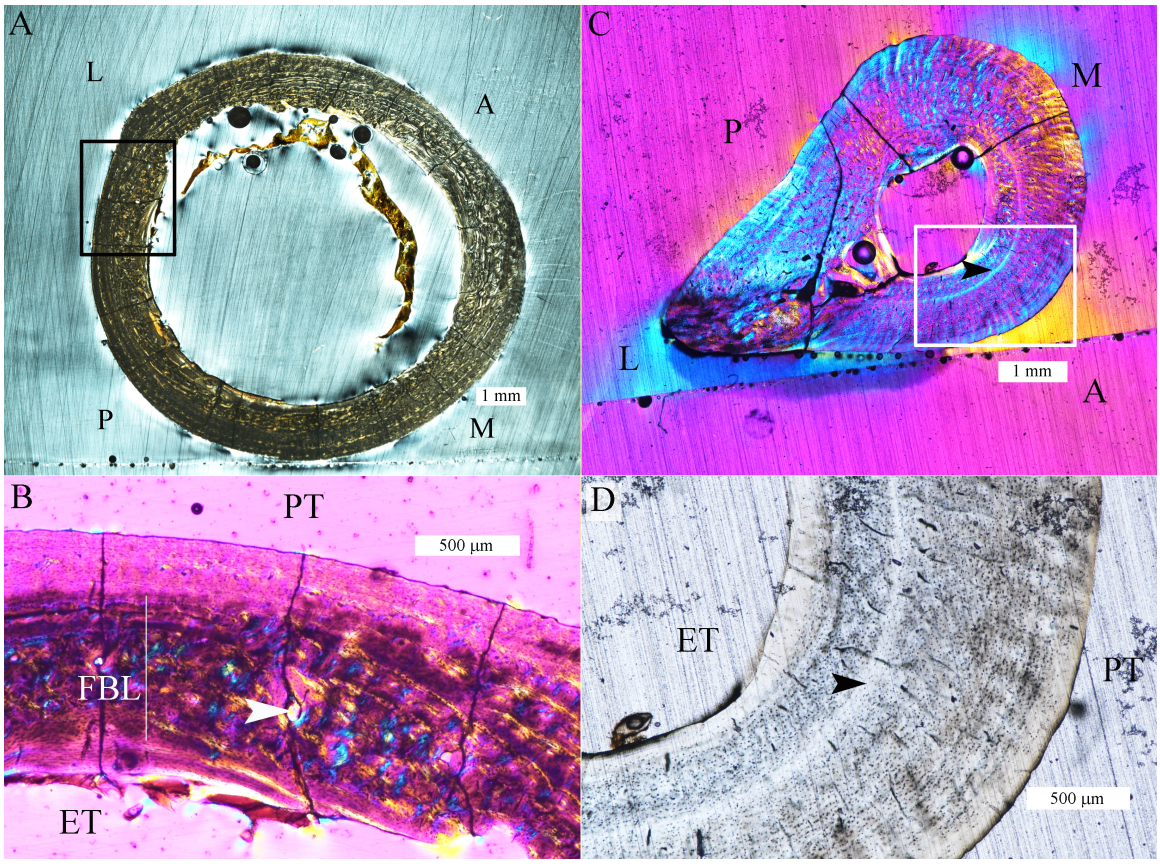


Fig.9 Microstructure of *Procyon lotor* and *Didelphis virginiana* humerii.

(A) This image shows the left humerus of the raccoon under cross-polarized light. The area of bone in the black box is magnified in B. (B) The left humerus of the raccoon under full wave plate light shows a high proportion of fibrolamellar bone (FBL). The white arrow points to a large vascular canal in the cortex. ET indicates the endosteal surface of the bone, while PT indicates the periosteal surface of the bone. (C) This image shows the opossum humerus under full wave plate light. The black arrow points to the only LAG seen in the opossum. The area of bone in the white box is shown at greater magnification in D. (D) This image shows the left opossum humerus under plane light. The black arrow points to the LAG seen in the humerus. The radial vascularization of the bone can be clearly seen here.

Radius

Figure 10 (A) and (B) show the raccoon radius under full wave plate light. There is a layer of lamellar bone around the endosteal surface of the right radius. On the anterior side the lamellar layer is thin. The rest of the cortex is fibrolamellar bone. There is longitudinal as well as laminar vascularization. On the posterior side the lamellar bone extends to nearly midway through the cortex. Fibrolamellar bone follows with reticular and some radial vascularization. There is a thin layer of avascular parallel-fibered bone on the periosteal surface. The lateral side is similar to the posterior side but has a larger proportion of radial vascularization. On the medial side the lamellar layer is thickest. Fibrolamellar bone with reticular vascularization makes up the rest of the cortex. There are large vascular canals seen throughout the cortex. One can be seen at the arrow in Fig. 10 (B).

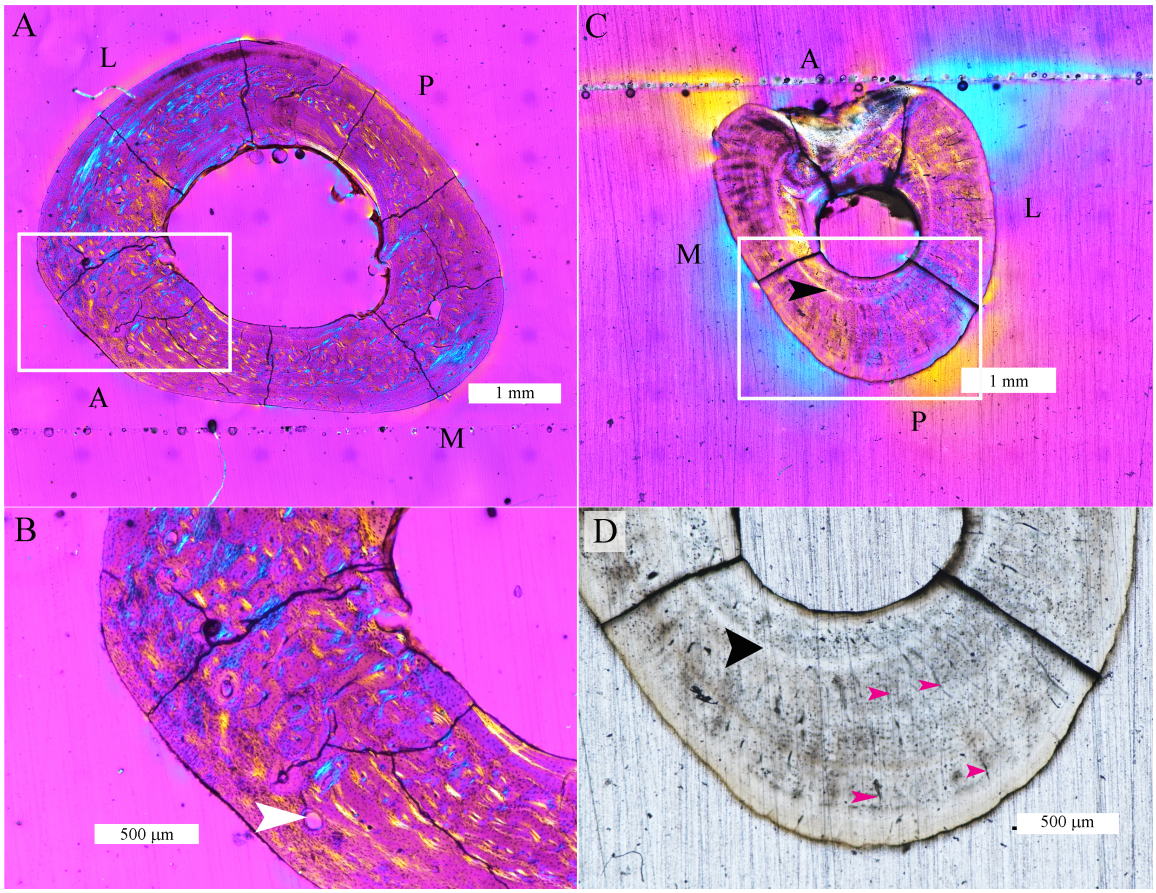


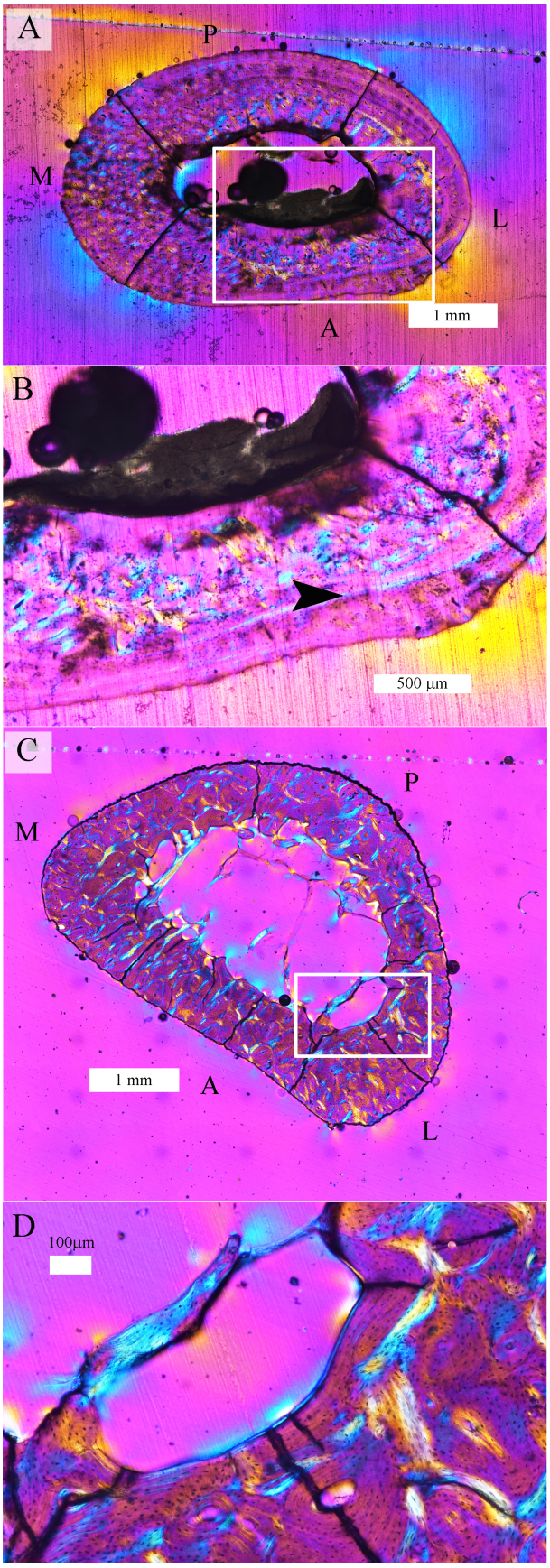
Fig.10 Microstructure of *Procyon lotor* and *Didelphis virginiana* radii.

(A) This image shows the left radius of the raccoon under full wave plate light. The cortex is mostly fibrolamellar bone with longitudinal vascularization, but the outer cortex shows some parallel-fibered bone as well. The area of bone in the white box is magnified in B. (B) This image shows the cortex of the left radius of the raccoon on the anterior side. The white arrow points to a large vascular canal. (C) This image shows the left radius of the opossum under full wave plate light. Bacterial degradation appears as fuzzy black areas on the slide. The black arrow points to the only LAG found in the opossum. The area of the bone in the white box is shown at greater magnification in D. (D) This image shows the left radius of the opossum under plane light. The black arrow points to a LAG and the magenta arrows point to several of the radial canals seen in the cortex.

Ulna

Figure 11 (C) and (D) show the raccoon ulna. There are sparse trabeculae composed of lamellar bone on the endosteal surface of the left ulna extending into the medullary cavity. The rest of the cortex is made of compact coarse cancellous bone with longitudinal vasculature. There are a few large vascular canals on the medial and lateral sides. Anteriorly, the right ulna has a thin layer of lamellar bone on the endosteal surface. The cortex is mainly composed of compact coarse cancellous bone. A resorption line and then a thin layer of fibrolamellar bone follow it. The posterior side exhibits a layer of lamellar bone on the endosteal surface followed by compact coarse cancellous bone. A resorption line and a band of fibrolamellar bone follow this. There is then a row of longitudinal vascular canals and finally a thin layer of lamellar bone along the periosteal edge of the cortex. The lateral side follows this pattern but the resorption line cannot be seen at the periosteal surface. The compact coarse cancellous bone that comprises most of the cortex is followed by a band of fibrolamellar bone that continues to the periosteal surface. There

are some loose trabeculae of lamellar avascular bone extending into the medullary cavity. Medially, the cortex is mostly composed of compact coarse cancellous bone. Close to the periosteal edge of the bone there is a resorption line followed by a thin strip of avascular fibrolamellar bone. The periosteal edge is rough and uneven.


A
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D
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Fig.11 Microstructure of *Procyon lotor* and *Didelphis virginiana* ulnae.

(A) This image shows the left ulna of the opossum under full wave plate light. The area of bone in the white box is magnified in B. (B) In this view of the left ulna of the opossum under full plate light the black arrow points to the only LAG found in the opossum skeletal elements. ET indicates the endosteal surface and PT indicates the periosteal surface. (C) The left ulna of the raccoon is shown here under full wave plate light. The cortex is mainly compact coarse cancellous bone, shown in the area in the white box, which is magnified in D. (D) The compact coarse cancellous bone that comprises the cortex is seen here.

Opossum

Humerus

Along the endosteal surface of the left humerus there is a layer of avascular lamellar bone. There are several large cavities on the lateral side of the bone surrounded by lamellar bone. The anterolateral side of the humerus has loose parallel-fibered bone from the inner cortex to mid-cortex with meager longitudinal vascularization. A LAG follows this layer (shown at arrow in Fig. 9 (C)) and the remaining the cortex is composed of parallel-fibered bone. Medially from the mid-cortex to outer cortex, there is loose parallel-fibered bone with minimal vasculature that is mostly longitudinal with some radial canals. Anteriorly, loose parallel-fibered bone comprises the cortex from the endosteal surface to mid-cortex and is followed by the continuing LAG and remaining cortex is parallel-fibered. It is nearly completely avascular, but there are a few radial canals. The cortex is thickest on the lateral side where three large canals are surrounded by lamellar bone. The cortex on the lateral side is mainly loose parallel-fibered bone. There is evidence of some bacterial degradation. The LAG can be followed around the bone on the anterior, medial and posterior sides. It is obscured on the lateral side, due to the remodeling associated with the crest in this location.

Radius

Figure 10 (C) shows the opossum radius in full plate light and (D) shows the same element under plane light. There is some bacterial degradation on the endosteal surface of the left radius especially on the anterolateral side. The medial side has a layer of avascular lamellar bone on the endosteal surface. From the inner cortex to mid-cortex the bone is loose parallel-fibered with sparse radial vascularization. This is followed by a LAG where growth has slowed or stopped for a period of time. This is the only LAG seen in the radius and it can be traced around the bone on the lateral, posterior and medial sides, although it is obscured on the anterior side due to remodeling. In Fig. 10 (C) a black arrow points to the LAG. The remaining cortex is parallel-fibered bone with radial vascularization. The outer cortex is parallel-fibered avascular bone. There is a depression on the lateral side of the bone on the periosteal surface. A layer of parallel-fibered bone comprises one third of the inner cortex. The remaining cortex is lamellar avascular bone. The right radius has extremely similar microstructure but more extensive bacterial degradation.

Ulna

Figure 11 (A) and (B) show the opossum ulna under full plate light. The endosteal surface of the left ulna is somewhat obscured by black blemishes due to bacterial degradation. There is no sign of an endosteal layer of lamellar bone. On the medial and posterior sides there is a layer of compact coarse cancellous bone from inner-cortex to mid-cortex. Medially, a LAG follows this and the remaining cortex is composed of parallel-fibered bone. The vasculature throughout the cortex is remarkably sparse and mostly longitudinal with some laminar canals. On the lateral and anterior side the entire cortex is loose parallel-fibered bone with little vasculature that is mostly longitudinal. The right ulna has a thin layer of avascular lamellar bone on the endosteal surface. The inner cortex is avascular parallel-fibered bone on the anterior side. There is

a layer of fibrolamellar bone in the mid-cortex with a few primary osteons and sparse longitudinal vascularization. This is followed by a LAG and another thin layer of lamellar bone. From mid-cortex to outer cortex the bone is parallel-fibered and mostly avascular with some laminar and radial vascularization in the mid-cortex. The posterior and medial sides do not exhibit a lamellar layer on the endosteal surface. The composition of the cortex on this side of the bone is loose parallel-fibered bone with a few primary osteons. The vascular canals are longitudinal and small but more regular than through the rest of the cortex. The LAG in this bone can be followed around the medial, posterior and lateral sides but is obscured on the anterior side by remodeling and perhaps some bacterial degradation.

CHAPTER IV

GENERAL TRENDS IN RACCOON HISTOLOGY

All bones in the raccoon skeletal series showed a layer of avascular lamellar endosteal bone. There were some sparse trabeculae in the ulna and dentary, but in general the mandibular canal is free of bone. The cortex is mainly fibrolamellar in the larger long bones, such as the femur and humerus, with an endosteal layer of lamellar bone and a layer of parallel-fibered bone at the outer cortex. The smaller long bones and the tibia exhibit a similar pattern, but have a cortex that is primarily compact coarse cancellous bone, rather than fibrolamellar. Enlow and Brown (1958) found that the cortex of adult raccoons was primarily composed of secondarily remodeled bone. No secondary remodeling is present, supporting that the specimen is a juvenile as evidenced by the lack of epiphyseal fusion, In the same study by Enlow and Brown, primary bone in adult *Procyon lotor* specimens was also found to exhibit reticular and radial vascularization, which was not confirmed by this study.

Several skeletal elements exhibited a high proportion of coarse cancellous bone including the femur, tibia, fibula, and ulna. This may have to do with the position of the point of least diaphyseal circumference of the raccoon bones, which was not found at the mid-diaphysis for any elements. During bone growth, cancellous bone located at the metaphysis becomes compact bone as the morphology of the bone changes and the metaphysis moves further down the lengthening bone (Enlow, 1963). The thin sections taken from the long bones may not have exhibited as much

compact coarse cancellous bone if the area of least circumference was located at the mid-diaphysis, as it is in many animals.

There are some secondary osteons in the areas of compact coarse cancellous bone; however, this is the only area in section where they occur. Several skeletal elements including the dentary, femur, tibia, humerus, and ulna contain resorption lines. The raccoon femur exhibits the highest proportion of fibrolamellar bone. The bone fibers are also more disorganized in the femur than in other elements containing fibrolamellar bone, such as the radius. This indicates that the raccoon femur most accurately records growth rate in *Procyon lotor* and is the best bone to sample for growth rate analysis.

GENERAL TRENDS IN OPOSSUM HISTOLOGY

All bones in the opossum skeletal series have a layer of avascular lamellar endosteal bone, although it is comparatively thinner than the layer of lamellar endosteal bone seen in the raccoon. Vascularization is sparse and sporadic, often most concentrated in the mid-cortex. Most vascular canals are radial, although some longitudinal can be seen as well. The humerus, ulna, radius, femur, and fibula all contain radial canals in parallel-fibered bone. The tibia is the only long bone that does not have any radial canals, but rather longitudinal vascularization. The tibia is also composed primarily of fibrolamellar bone. In general, there is low osteocyte density throughout the cortex of all bones. The cortex of all possum bones excluding the tibia are mostly formed of parallel-fibered bone that is often avascular, or meagerly vascularized. The exception is in the dentary, where vascularization is denser than seen in the other skeletal elements. Many of the possum skeletal elements show black opaque spots that are due to bacterial degradation of the bone. A single LAG is seen in the humerus, radius and ulna, so the opossum was at least one year old at the time of death.

This study supports the work done by Kolb et al. on *D. albiventris* and *L. crassicaudata*, finding a prevalence of parallel-fibered tissue in the cortex and a layer of lamellar bone on the endosteal circumference. In *Lutreolina* they found radial, longitudinal and oblique vascularization, whereas this study identified mainly radial vascularization with some longitudinal canals. Werning (2013) found a similar histological pattern in placentals and marsupials of the same size and postulates that a cortex composed of nearly avascular lamellar bone is a plesiomorphic character for small therians. This study did not find similar results. The raccoon, a placental mammal, and the opossum, a marsupial, are both similarly sized, yet have substantially different histological patterns. This may be a difference between these two species, rather than between placentals and other mammals. Further studies are needed to continue answering this question.

COMPARISON OF HISTOLOGY

In general, the bone tissue of the opossum suggests slower growth. There is a greater proportion of parallel-fibered bone, as opposed to the greater proportion of fibrolamellar bone seen in the raccoon. Fibrolamellar bone and parallel-fibered bone have been shown to have overlapping growth rates. Woven bone in the raccoon was found to have laminar, longitudinal and plexiform vascularity which has been shown to have growth rates between 10 μm/day – 39.5 μm/day, 4.8 μm/day – 50 μm/day, and 5 μm/day – 15 μm/day respectively (Castanet et al. 1996, 2000). Although the rate of tissue deposition in the opossum and raccoon may have been similar, there is a striking difference in the vascularization patterns between the two animals. Vascularization in all skeletal elements of the opossum is remarkably sparse and composed predominantly by radial canals, with occasional longitudinal canals. This is unusual as radial vascularization is rarely seen in slowly growing tissue such as parallel-fibered bone, and is usually seen in fibrolamellar bone that is growing rapidly (Huttenlocker, Woodward and Hall, 2013). Vascularization is denser in the raccoon and mostly longitudinal, but with some laminar

and plexiform canals. Both animals show compact coarse cancellous bone in several of the sectioned elements, most likely due to the area of the bone that was sectioned. Although histological sections are often made using tissue taken from the mid-diaphysis to avoid areas of bone more likely to be remodeled (Chinsamy-Turan 2011, Francillon-Vieillot 1990, Padian and Lamm 2013), the areas of least circumference in the majority of the opossum and raccoon long bones were found close to the epiphyses of the bones, which may explain the preponderance of compact coarse cancellous bone. During bone formation, the epiphyseal region of the bone is composed of spongy trabeculated bone. As the bone increases in length, this area is closer to the mid diaphysis of the bone and is remodeled so the cortex becomes compact bone. The remnants of earlier primary trabeculae can still be seen in the cortex and give compact coarse cancellous bone a distinctive fiber orientation pattern. Both dentaries show evidence of rapid growth and lines of resorption, but no LAGs. The opossum has one LAG in the humerus, radius and ulna, which is not continuous due to remodeling. Osteocyte lacunae are dense and there is fibrolamellar bone on the ventral side of both dentaries with primary osteocytes.

The osteohistology of these two animals is quite different. Both occupy a similar range in North America as well as a similar ecological niche. Raccoons appear to be growing to a mature size at a rapid rate of growth, while the large proportion of parallel-fibered bone in the cortex of the opossum long bones indicate that they reach a comparable size (the opossum femur is 7.1cm long, while the raccoon femur is 11.2 cm long) at a lower rate of growth. Opossums have a lower metabolism (0.15 ml O_2/g of body weight/hr) than raccoons (0.54 ml O_2/g of body weight/hr), which allows them to conserve energy and utilize fewer resources. The opossum is the only marsupial that has successfully transitioned to North America and shares the low metabolism of others in Marsupialia. Most procyonids also have low metabolic rates (Mugaas 1993), a character which is basal to the family. A high metabolic rate is an apomorphy for *Procyon lotor*, allowing the raccoon to expand its habitation northward. However, both species now inhabit the northern

areas of North America, possibly due to rising temperatures of recent years. Future histological research on fossil procyonids and marsupials may confirm a phylogenetic constraint on osteohistology resulting in different patterns in the two groups. Alternately, similar osteohistological patterns might reveal the influence of a historically tropical or subtropical environment on procyonid and marsupial histology. This study does support Padian (2013), which observed that phylogenetic signal influences bone microstructure, but further histological research is needed to determine whether this is seen throughout the marsupial and procyonid lineages.

Osteohistological samples are usually taken from the mid-diaphysis of long bones in an attempt to avoid areas of the bone that have been remodeled due to bony processes or the biomechanical influence of muscle attachment sites (Chinsamy-Turan 2011, Francillon-Vieillot 1990, Padian and Lamm 2013). This is usually the area of least circumference, however in the opossum and the raccoon, the area of least circumference was found to be closer to the epiphyseal end of the bone than the mid-diaphysis. The area of least circumference is also where one would expect remodeling due to growth and shape change of the bone to be lowest because less bone is present overall. The location in the opossum and raccoon may be due to similar biomechanical requirements of these two arboreal animals that spend much of their time climbing which necessitates a greater ability to maneuver their limbs. In many animals non-weight bearing bones are usually sectioned for research as these are not thought to be under the same biomechanical stresses as weight-bearing elements (Stein and Sander 2009, Waskow and Sander 2014). Although weight-bearing bones give an accurate depiction of growth rate, nonweight-bearing bones are thought to most accurately record the number of LAGs and thus the age of an animal. In the opossum, the single LAG found in the animal was most completely recorded in the radius, continuing nearly entirely around the circumference of the cortex, suggesting that this may be the most opportune bone to sample when analyzing age in the opossum. No significant fibrolamellar

tissue was found in the opossum, making it difficult to determine the best bone to section when determining growth rate. The femur of the raccoon had the highest percentage of fibrolamellar bone and is most likely the best element to sample when analyzing growth rate of *Procyon lotor*. None of the raccoon bones recorded any LAGs, so this study was unable to determine the best bone to section when analyzing raccoon skeletochronology.

The two animals assessed in this study were most likely juveniles, as the epiphyses were un-fused. Although most research on epiphyseal fusion has occurred in humans, studies of other smalls mammals have found the timing of fusion to vary, but typically occurs after 1 year of age has been attained (Sumner-Smith 1966). *Didelphis albiventris*, the white-eared opossum has been found to exhibit LAGs, and raccoons have been found to deposit annual LAGs like most carnivorans (Kolb et al. 2015). Many modern animals have been shown to form LAGs annually (Castanet 2004, de Buffrénil 2000, Köhler 2012), demonstrating that the opossum is at least one year old while the raccoon is less than a year old. Modern raccoons and opossums have similar lifespans in the wild, with raccoons living 2 – 4 years (Austad 1997) and opossums surviving an average of two years (Johnson-Delaney 2014). As these animals inhabit similar, even overlapping environments, further research into the causes of death could illuminate the connection between a slower rate of growth in opossums and an earlier average age of demise. It seems counterintuitive that an animal growing more slowly would die at an earlier age, although perhaps the slow rate of growth does not affect the age of maturity and is mainly an adaptation to preserve energy and exploit resources efficiently.

Further research into the histology of extant animals is needed, including an ontogenetic survey of vertebrates. This would supplement early comprehensive studies of bone histology that often only encompassed adult specimens (Enlow 1958). Another interesting direction for research is the location of sectioning that provides the most accurate data for an animal. In many mammals and dinosaurs, the area of least circumference in a long bone is located at the mid-diaphysis.

However, in both the opossum and the raccoon, the location of least circumference was found to be close to the epiphyses of most bones. It is possible that this character is alike in both animals because they have similar biomechanical requirements for their omnivorous and arboreal lifestyles. Perhaps the similarity of their locomotion has resulted in a similar morphological compensation. This could be studied by creating serial thin sections of a juvenile, young adult, and adult opossum and raccoon to compare the record of growth in different locations of the long bones at different ontogenetic stages.

As previously noted in this study, a considerable amount of research has been conducted hypothesizing on the metabolic physiology of extinct animals. This study shows that mesopredators of similar sizes may have different histological patterns, yet share a category of metabolic physiology, in this case endothermy. When interpreting the fossil record, caution must be taken in proposing metabolic strategies for animals based on histological patterns. For example, the American alligator, histologically sampled and analyzed in Woodward et al. (2014), exhibits bone microstructure that is similar to *D. virginiana* in several ways. Skeletal elements of the alligator exhibit LAGs, parallel-fibered tissue and low vascularization. It is easy to see how two extinct animals with similar microstructure could be hypothesized to have similar metabolic physiologies, when one is endothermic and the other ectothermic. The diversity of growth rates seen in different bone tissue types between species of extant animals most likely existed in extinct animals as well. The bone microstructure of mesopredators from the fossil record must be examined under guidance from osteohistological data of modern analogues. For example, the fossil marsupial *Peradectes minor* is an extinct relative of *Didelphis virginiana* that lived 66 – 63 million years ago and has not previously been histologically sectioned. The extinct procyonid *Cyonasua* lived 7.3 – 5 million years ago and is another animal from the fossil record that has not yet been histologically sampled. The current study could provide insight into the physiologies of extinct animals such as these. As metabolic rate is a plastic character (Mugaas 1993),

osteohistology can assist in detailing the physiological profiles of extinct taxa. This is another reason to continue the work of increasing the database of qualitative descriptions of the osteohistology of modern taxa.

CHAPTER V

CONCLUSION

The fossil record presents many challenges to paleontologists aiming to better understand animals, plants, and ecologies long extinct. One way to illuminate transitory physiological aspects of extinct taxa is to study bone microstructure. The only way to assemble a complete picture of extinct taxa using fossil bones is to incorporate an intimate understanding of the bone biology of extant animals. To that end, this study contributes to the momentous undertaking of compiling an extensive osteohistology database for modern vertebrates.

The patterns of bone microstructure examined in this study contribute to the continuing research on extinct mesopredators. Further histological work may also benefit from the observation that the femur seems to be the best element to section in raccoons to analyze growth rate, while the radius is better at providing a complete LAG count in opossums. It has previously been asserted that non-weight bearing bones are the best choice for histological sectioning when analyzing skeletochronology, which does seem to hold true for the opossum. The contrast in histological narrative recorded by two similar animals cautions us against inferring animals are endothermic or ectothermic solely based on particularly slow or fast- growing bone. After all, these two animals have markedly different metabolisms but are both endothermic.

Although these findings may seem to indicate that phylogenetic constraints are acting upon bone microstructure more stringently than environment, it is worth noting that *P. lotor* has a much higher metabolic rate than other procyonids that have historically been confined to tropical and subtropical areas, much like the opossum, which only migrated to North America during the Miocene. Comparing the bone microstructure of a marsupial and a procyonid restricted to tropical zones might yield differing histological results from the present study.

The study of fossil tetrapods will only be enhanced as scientists continue to catalogue and quantify the astounding diversity and variety of extant vertebrates. Histology is an excellent way to gain insight into the physiology of extinct animals, a trait that is not directly chronicled in the fossil record. As the biology of extant animals is more intimately understood, the field of paleontology can build an increasingly complex picture and comprehension of the history of life on earth.

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